REMARKS/ARGUMENTS

By the present amendment, claims 1 and 17 have been amended as described below. The amendments to the clams have been made without prejudice and without acquiescing to any of the Examiner's objections. Applicant reserves the right to pursue any of the deleted subject matter in a further continuation, continuation-in-part or divisional application. The amendment does not contain new matter and its entry is respectfully requested.

The Official Action dated December 29, 2005 has been carefully considered. It is believed that the amended specification and the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

Applicant thanks the Examiner for the courtesies extended during the interview that was held on May 2, 2006.

Claim Objections

The Examiner has objected to claims 23 because the status identifier of the claims is improper according to 37 CFR 1.121. In response, the status identifier of the claims has been corrected.

35 U.S.C. §112 - second paragraph

The Examiner has objected to claims 1, 3, 5-17 and 21-23 because in the claims are drawn to a method of producing chymosin by a method of claim 1, wherein chymosin is purified to homogeneity but the specification does not define "homogeneity". In response, claims 1 and 17 have been modified to replace "such that said chymosin is purified to homogeneity" with "such that said chymosin is purified". Support for this

amendment is found in Example 5, "Purification of chymosin from transgenic Brassica napus seed".

In view of the foregoing, we respectfully request that the objections to the claims under 35 U.S.C. 112, second paragraph be withdrawn.

35 U.S.C. §112 - first paragraph

The Examiner has objected to claims 1, 3, 5-17 and 21-23 under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement. Specifically, the subject matter a "chymosin is purified to homogeneity" is rejected as pertaining to new matter. In response, claims 1 and 17 have been modified to replace "such that said chymosin is purified to homogeneity" with "such that said chymosin is purified". Support for this amendment is found in Example 5, "Purification of chymosin from transgenic Brassica napus seed".

In view of the foregoing, we respectfully request that the objection to the claims under 35 USC 112, first paragraph be withdrawn.

35 U.S.C. §103

The Examiner has objected to claims 1-8, 10, 11 and 13-23 under 35 USC §103(a) as being unpatentable over Willmitzer et al. (WO 92/01042) in view of Applicant's admitted prior art. We respectfully disagree with the Examiner for the reasons that follow.

In order to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be a suggestion or motivation to modify the reference. Second, there must be a reasonable expectation of success. Finally, the prior art reference must teach or suggest all of the claim limitations. None of these are met by Willmitzer et al. or Applicant's admitted prior art.

Willmitzer et al. generally teaches that industrial enzymes, such as chymosin, can be prepared in plants. The present claims relate to the production and isolation of chymosin from plant seeds. Willmitzer et al. does not produce or isolate chymosin from plant seeds and does not teach how to isolate chymosin from plant seeds. Further, there is nothing in Willmitzer et al. that would motivate one of skill to develop an isolation method as described in the claims.

With respect to motivation, the Examiner refers to the Abstract and page 9, line 32 to page 10, line 8 of Willmitzer et al. While the Abstract of Willmitzer does indicate that the enzyme may be recovered from the plant, p. 9, line 32, bridging p. 10, line 8 is not referring to recovering a enzyme from a plant but referring to the fact that "seeds should be tested to assure that the new genetic trait has been inherited in a stable Mendelian fashion." The Examiner has also indicated that Willmitzer clearly was recovering chymosin using a polyacrylamide gel protein binding resin. We respectfully submit that polyacrylamide gel electrophoresis (PAGE) is used to separate proteins on the basis of their size and charge through (i.e. not by binding protein) a polyacrylamide gel matrix so that individual proteins bands can be detected using for example Coomassie Blue protein stain or using a specific immunoglobulin. Importantly, PAGE is a destructive method and renders the protein non-functional and not useful for commercial purposes. One skilled in the art would realize that Willmitzer is using PAGE to detect an individual protein in a complex mixture of proteins and not using it to recover and purify the chymosin away from a total extract comprising the chymosin. As specifically described in Willmitzer on page 12, lines 18-32, they are describing the "Detection of proteins" (emphasis added) (page 12, line 18) and indicate that "the amount of a specific protein present in total extracts was estimated ..." (emphasis added). Therefore, Willmitzer has not described the purification of chymosin from seed tissue.

With respect to a reasonable expectation of success, Willmitzer only discloses detecting chymosin in leaves. One skilled in the art would not necessarily expect that the method for purifying a protein from leaves would be successful when purifying the same protein from seeds comprising an oil fraction. As disclosed in the current application (page 22,

lines 1-16) it is desirable to remove the oil fraction when chymosin is produced in seed comprising a relatively high oil content. As discussed previously in the May 27, 2004 response, the purification of recombination proteins from oil seeds was difficult due to the presence of large quantities of oil which would make the subsequent purification steps problematic. The art-recognized solution to the problem was to extract the oil using conventional hexane extraction procedures. This is discussed in greater detail below. However, the use of hexane or other organics solvents to extract proteins was not desirable due to the denaturant property of such solvents. Therefore, a person skilled in the art would not have a reasonable expectation of success purifying chymosin from seed comprising an oil fraction using either methods for the purification of proteins from leaves or the use of conventional hexane extraction procedures. Therefore, the present invention provides a solution which is not obvious in light of the prior art.

Finally, the prior art reference must teach or suggest all of the claim limitations. The current claims are limited to a specific method for the isolation of chymosin from a seed comprising an oil fraction. This method involves, (i) crushing the plant seed to obtain crushed plant seed; (ii) fractionating the crushed plant seed into an oil fraction, aqueous fraction and a fraction comprising insoluble material; (iii) contacting the aqueous fraction with a protein binding resin; and (iv) recovering chymosin from the protein binding resin such that said chymosin is purified.

At the bottom of page 4 of the office action, the Examiner states that (a) all plants have oils and (b) the method of isolating the protein is taught by the prior art. We respectfully submit that even though plants do contain some level of oil, not all plant tissue or even all plant seeds, comprise an oil fraction that would make purification of a protein problematic. Further, Willmitzer does not teach all of the method steps of the invention. While Willmitzer discloses a method for the extraction of protein from transgenic plants (page 12, lines 8-16), there are many steps that are missing from Willmitzer. In Willmitzer the plant tissue is homogenized in a protein extraction buffer, the plant tissue is filtered through cheese cloth and insoluble components are pelleted by centrifugation. Nowhere in Willmitzer is it disclosed that the crushed plant tissue is fractionated into an

oil fraction, aqueous fraction and a fraction comprising insoluble material. Willmitzer discloses fractionating the tissue into soluble and insoluble components. Furthermore, Willmitzer does not recover chymosin from a protein binding resin as discussed above. Willmitzer is using polyacrylamide gel electrophoresis to detect and visualize the amount of a specific protein present in total extracts (see page 12, lines 29 and 30). As discussed above, polyacrylamide gel works as a sieve to crudely separate proteins based on mass and it does not involve binding the proteins. This is an analytical method used merely to determine the presence and size of a protein. Therefore, we respectfully submit that Willmitzer is using denaturing gel electrophoresis and Western blot analysis for the immunological detection of proteins and is not recovering the chymosin from a protein binding resin. Therefore, Willmitzer is not teaching a method of protein isolation using a protein binding resin.

In the office action dated June 28, 2005, the Examiner stated that the claims were obvious over Willmitzer et al. "in view of Applicant's admitted prior art". Applicant's admitted prior art can be found in the Background of the Invention on pages 1-3 of the application as filed. None of these references discussed would render the present invention obvious. Importantly, the Background discloses Willmitzer et al. and highlights that Willmitzer et al. only prepares chymosin in leaves and at much reduced levels compared to the present invention where chymosin is prepared in seed.

At the interview, Primary Examiner T. Bui suggested that "Applicant's admitted art" included the discussion of the isolation of chymosin from seed on pages 20-25 of the application. Specifically, Examiner Bui noted that the methods described are known in the art. We respectfully submit that the Examiner appears to be assessing all of the method steps in the claims separately rather than assessing the method as a whole. The entire method described in independent claims 1 and 17 is both novel and inventive over the prior art. Pages 20-25 teach how one of skill in the art can carry out the isolation steps of the method described in step (d) of claim 1 or step (f) of claim 17. However, Applicant is not attempting to claim any one of these methods (e.g.

centrifugation or column chromatography) on their own. Applicant is preparing a novel and inventive method for the production and isolation of chymosin from plant seed.

In view of the foregoing, there is no basis for rejecting the claims based on "Applicant's admitted prior art".

In fact, the state of the art at the time of the invention supports the inventiveness of the claims. At the time of the invention and even for years after the present application was filed in August 2000, it was abundantly clear that the recovery and purification of recombinant proteins from plants is a distinct challenge from the synthesis of recombinant proteins in plants. For example, a 1999 review article in Nature Biotechnology by Borisjuk et al. (Nature Biotechnology, vol. 17, May 1999, page 466-469, copy enclosed) opens with the statement:

> ***"The large-scale production of recombinant proteins in plants is limited by relatively low yields and difficulties in extraction and purification."***

In addition, a paper by Komarnytsky et al. (Plant Physiology, November 2000, vol. 124, page 927-933, copy enclosed) states at page 927 that:

> ***"the high cost of protein extraction and purification from biochemically complex plant tissues is an important obstacle for the large-scale protein production in plants."***

Further, in a review article by Fischer et al. (Current Opinion in Plant Biology 2004, 7: 152-158, copy enclosed) published in 2004 which was many years after the present application was filed the authors state that:

> ***"Current limitations of plant bioreactor technology include the low yields that are achieved for many proteins (which are often caused***

> ***by poor protein stability), difficulties with downstream processing (leading to inconsistent product quality)...."***

In yet another 2004 review article (Biotechnol. Prog. 2004, 220, 1001-1014, copy enclosed) Menkhaus et al. state that:

> ***"...94% of the annual operating costs (AOC) for producing and purifying β-glucuronidase (GUS) from transgenic maize were sustained during seed fractionation (6% of AOC), protein extraction (40% of AOC), and purification (48% of AOC). Therefore, research to develop and improve downstream processing methods could be foreseen to greatly reduce the overall costs associated with many products and perhaps to dictate (along with expression levels) the future feasibility for commercial application."***

Therefore, it is clear that at the time of the invention, the art recognized that isolating and purifying recombinant proteins from plants in commercial quantities was a problem. It is also clear from the literature that when dealing with the isolation of proteins from seeds that contain a high oil content, at the time of the invention the only solution was to use an organic solvent to extract the oil. For example, Zhang and Glatz (Biotechnol. Prog., 1999, 15, 12-18, copy enclosed) describes the purification of lysozyme from canola extracts and describes that the oil is removed with cold hexane extractions (page 13). Kusnadi et al. (Biotechnology Bioengineering, vol. 60, no. 1, October 5, 1998, copy enclosed) describes the isolation of β-glucuronidase (GUS) from corn using hexane extraction. Importantly, in the references that Applicant has reviewed that were published prior to 2000, no attempt was made to isolate or purify the recombinant protein from the plant in the majority of the cases. Therefore, at the time of the invention, there was nothing in the state of the art that would lead one of skill in the art to the present invention.

With respect, the Examiner has not met the criteria for establishing a prima facie case of obviousness. In fact, the literature as a whole teaches away from the present invention by only providing one solution (i.e. hexane extraction) for the removal of oil in a separation process. Importantly, none of the literature describes a method for the purification and recovery of **chymosin** from plant seeds that contain an oil fraction. Applicant has developed a method that is useful in recovering commercially useful quantities of chymosin from plant seed. This method was clearly not taught or suggested in the literature at the time of the invention.

Applicant submitted a Declaration under 37 CFR § 1.132 by David Dennis with the response filed May 27, 2004. Dr. Dennis is a researcher with over 40 years experience as a plant biochemist and currently the President of a plant biotechnology company. It appears that the Examiner has dismissed the Declaration on the basis that Dr. Dennis uses the term "homogeneity" which is not in the claims. We submit that the language used by Dr. Dennis is consistent with the claims which state that the chymosin is purified. One of skill in the art would understand that a purified protein is homogenous. In this regard we refer to Guidelines for Protein Purification (http://www-ccs.ki.ku.dk/phdcourse/GuidelinesForProteinPreparation.html) wherein it states that a purified protein will be as "homogenous as possible". We also refer to the definition of "pure" which states "having a homogenous or uniform composition" (http://www.answers.com/topic/pure). Therefore, since there is a nexus between the evidence provided in the Dennis Declaration and the claimed invention, the Declaration is entitled to substantial weight in the rebuttal arguments against the obviousness rejection. (See MPEP 2144.08). We respectfully request that the Declaration by Dr. Dennis be reconsidered by the Examiner. Specifically, we refer to paragraph 12 of the Dennis declaration which states:

> ***"The methodologies of protein purification have to be developed and established for each protein that is purified. This is especially true for the purification of chymosin from plant seeds where pure active protein is required in large amounts. Applicant has developed a***

methodology that permits purification of chymosin from plant seeds. Applicant has achieved purification of chymosin from plant seed by a methodology which involves the steps of fractionating crushed seed into an oil fraction, an aqueous fraction and a fraction comprising insoluble material and then subsequently contacting the aqueous fraction containing the chymosin with a protein binding resin. These method steps are not obvious in light of Willmitzer."

In summary, we respectfully submit that the Examiner has not established a *prima facie* case of obviousness for the following reasons:

- **Willmitzer does not isolate and purify chymosin from seed.**

- **Willmitzer does not teach or suggest how to isolate and purify chymosin from seed.**

- **No prior art, including the Applicant's admitted prior art, teaches or suggests how to isolate and purify chymosin from seed.**

- **The state of the art at the time of the invention taught hexane extraction of proteins from oil seeds which is not desirable.**

- **Dr. Dennis, an expert in this field, confirms that the method claimed in the present application is unobvious over Willmitzer and the state of the art.**

In view of the foregoing, we respectfully request that all of the objections to the claims under 35 U.S.C. §103 be withdrawn.

The Commissioner is hereby authorized to charge any deficiency in fees (including any claim fees) or credit any overpayment to our Deposit Account No. 02-2095.

In view of the foregoing, we submit that the application is in order for allowance and an early indication to that effect would be greatly appreciated.

Respectfully submitted,

GIJS VAN ROOIJEN ET AL.

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